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VARIATION IN *COLLETOTRICHUM GLOEOSPORIOIDES* ISOLATES FROM BANANA

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Abstract: Isolates of *Colletotrichum gloeosporioides* obtained from anthracnose lesions on fruits of the banana cultivars, anamalu, embul, embon, kolikuttu and kochchikehel were examined for differences in cultural characteristics and pectic enzyme secretion. Significant differences were observed in the growth rate and in the lengths of conidia among some isolates. Sporulation differed significantly among all isolates. All isolates secreted a single form of similar molecular size of each of the pectic enzymes polygalacturonase and pectin lyase.

Key words: Banana, Colletotrichum gloeosporioides, pectic enzymes.

INTRODUCTION

The fungus *Colletorichum gloeosporioides* causes the anthracnose disease of banana fruits.[1] The disease affects different cultivars of banana and results in the formation of spreading lesions on infected fruits. *C. gloeosporioides* also causes infections in many other economically important plants such as *Hevea brasiliensis, Stylosanthus*, wheat, alfalfa and orchard grass. Each of these plants are infected by many isolates of the fungus and differences among the isolates have been established.[2,3] A knowledge of such variations in pathogenic fungi is useful for disease control, development of new cultivars and in cultivation programmes. Hence, this study was undertaken to determine whether differences exist among isolates of *C. gloeosporioides* obtained from naturally infected fruits of different banana cultivars. The cultural behaviour of the isolates and the characteristics of secreted pectolytic enzymes were examined.

METHODS AND MATERIALS

Organism: Colletotrichum gloeosporioides was isolated from naturally infected anthracnose lesions on banana fruits. All cultures used in the experiments were derived from single conidia and maintained on potato dextrose agar (PDA) at 4°C. The isolates were Kch (from kochchikehel), Kkt (from kolikuttu), Emn (from embon), Eml (from embul) and Anm (from anamalu). The identity of all the isolates were confirmed by the International Mycological Institute, London.

Growth Media: (i) Solid Media: PDA, Czapek-Dox agar (CDA) and Malt Extract Agar (MEA) were used. Fifteen ml of each of the medium was dispensed into 9 cm diameter petri dishes.

(ii) Liquid medium: Ammonium tartrate liquid medium[4] with citrus pectin as the major source of carbon was used for studies on enzyme secretion. Twenty five ml of the liquid medium was dispensed into 150 ml Erlenmyer flasks.

Inoculation and Incubation: The solid media were inoculated at the centre with 0.5 cm diameter plugs taken from the periphery of 7-day old cultures of the different fungal isolates growing on PDA at 25°C. Unless otherwise stated, all inoculated cultures were incubated at 25°C. The liquid media were inoculated with two 0.5 cm diameter plugs taken from cultures in the manner described above, and incubated at 25°C without shaking.

Assessment of Growth: The growth of each isolate on PDA, CDA and MEA were assessed by measuring the colony radius along two axes at right angles 48, 72, 96, 120, 144, 168 and 192 h following inoculation.[5]

Determination of Conidia Concentration and Size : The isolates were grown on PDA at 25°C for 7 days. At the end of the 7 day period the colonies were flooded with 10 ml sterile distilled water and the colony surface was mechanically disturbed to suspend the conidia. Thereafter, the conidial suspension was filtered through two layers of muslin cloth. The conidia concentration in the resulting filtrate was determined using a haemocytometer.[3] In each sample the length and width of 100 conidia was also measured.

Determination of Enzyme Activity: The liquid media were harvested 7d after inoculation by filtration through Whatman's no 1 filter paper. The culture filtrates were dialysed against distilled water at 4°C for 48h and concentrated ten fold by freeze drying. The dialysed concentrated culture filtrates were used to determine enzyme activity.

(a) Polygalacturonase (PG) : PG activity was determined using the agar plate method and by the release of reducing sugars from 0.1% (w/v) solutions of polygalacturonic acid in 0.1M sodium acetate buffer (pH 5.0).[2] In the agar plate method a sodium polypectate gel was used and activities were determined relative to an aqueous solution of 10M pectinol (Rohm and Hass, USA).

(b) Pectin lyase (PL) : PL was assayed using the thiobarbituric acid (TBA) method.[6]

Determination of Molecular Weights: Molecular weights were determined by gel filtration[7] using a column of Sephadex G-100 (80x2.6 cm^2) eluted with pH 7.6 buffer containing 0.05M Tris-HCl, 0.1M KCl and 0.2M NaN_3. Bovine serum albumin (66,000), ovalbumin (45,000), pepsin (35,000) and cytochrome-c (12,400), (Sigma, USA), were used as molecular weight markers.

RESULTS

Growth of the Isolates

The growth of all isolates were best on PDA (Table 1). In this medium the growth rates of the isolates Anm and Eml differed significantly from each other and from those of Kkt, Emn and Kch. On the MEA medium the growth of the isolate Eml differed significantly from all the other four isolates. Based on the observed growth rates, on CDA the isolates can be separated into four categories; isolates Eml; Anm; Kch; Kkt together with Emn (Table 1).

Table 1: Growth rate (cm/day) of isolates of *Colletotrichum gloeosporioides* on different media.

Isolate	Growth media*		
	PDA	MEA	CDA
Anm	0.67 ±.02[a]	0.60 ±.01[d]	0.45 ±.02[f]
Kkt	0.92 ±.03[b]	0.62 ±.02[d]	0.57 ±.03[g]
Emn	0.93 ±.02[b]	0.63 ±.03[d]	0.61 ±.01[g]
Eml	0.80 ±.01[c]	0.77 ±.02[e]	0.78 ±.01[h]
Kch	0.94 ±.02[b]	0.62 ±.03[d]	0.69 ±.01[i]

*Mean of 5 replicates ± standard errors. Values followed by the same letter in a column are not significantly different at P = 0.05 (Duncan's multiple range test). CDA - Czapek-dox agar; MEA - Malt extract agar; PDA - Potato dextrose agar.

Sporulation

Sporulation was very profuse in isolate Anm and lowest in the isolate Kch (Table 2). The sporulation of all five isolates differed significantly from each other.

Table 2: Sporulation of isolates of *C. gloeosporioides* grown on PDA for 7 days at 25°C.

Isolate	Conidia concentration* Conidia/ml x 10^5
Kch	10.75 ±1.2[a]
Kkt	31.95 ±3.2[b]
Emn	24.75 ±2.1[c]
Eml	45.65 ±2.8[d]
Anm	61.95 ±1.7[e]

*Mean of 5 replicates. ± Standard errors. Values followed by the same letter in a column are not significantly different at P=0.05 (Duncan's multiple range test). PDA-Potato dextrose agar.

Conidia Dimensions

The breadth of conidia of all isolates were similar. But, the conidial lengths of the isolates Kkt, Eml and Anm differed significantly (Table 3). No significant difference existed between conidial lengths of isolates Kch and Emn. The conidia were longest in Kkt (18 μm) and shortest in Eml (10 μm).

Table 3: Dimension of conidia of isolates of *Colletotrichum gloeosporioides* grown on PDA for 7 days at 25°C.

Isolate	Conidia length (μm)	Conidia breadth (μm)
Kch	13.8 ±.2[a]	3.8 ±.1[a]
Kkt	18.6 ±.3[b]	4.0 ±.2[a]
Emn	13.9 ±.1[a]	4.0 ±.3[a]
Eml	8.9 ±.2[c]	3.9 ±.1[a]
Anm	16.1 ±.1[d]	3.8 ±.2[a]

*Mean of 5 replicates (in each experiment 100 conidia were measured). ± standard errors. Values followed by the same letter in a column are not significantly dfferent at p=0.05 (Duncan's multiple range test). PDA -potato dextrose agars.

Enzyme Production: All isolates produced single forms of the pectic enzymes PG and PL. The molecular weights of PG and PL respectively from the isolates were: Kch (24,000 ±750; 33,000 ±250), Kkt (23,500 ±500; 34,500 ±500), Emn (25,500 ±750; 32,500 ±750), Eml (24,500 ±250; 33,000 ±750) and Anm (23,000 ±750; 32,000 ±500). All values are the mean of three replicates.

DISCUSSION

Significant differences existed among the different isolates with respect to their rates of growth, length of conidia and sporulation. All the differences observed between the isolates were stable. Such differences have also been reported for the *Colletotrichum gloeosporioides* isolates from rubber and *Stylosanthus*.[2,3]

The rates of growth of banana isolates on PDA, the range of conidial lengths and breadths reported here agree with those reported for the isolates from rubber and *Stylosanthus*. But, the sporulation is less than that of the rubber isolates.[2,3]

There was no difference among the isolates in the secretion of the two pectic enzymes, PG and PL. Molecular weight determinations suggest that the PGs and PLs of all isolates are similar. The molecular weights of PG reported here for the *C. gloeosporioides* isolates agree with those reported for the *C. gloeosporioides* isolates from rubber[2,8] but not *Poria hypolateritia* from tea plants.[9] The molecular weights of PL also agree with the molecular weights of several other pathogenic fungi but differ from the PL of the rubber isolate.[2,8,10] The molecular weight reported for the PL from rubber isolates were 4,150.[2,8]

Among the isolates examined, the isolate Eml differed significantly from the rest of the tested isolates in its growth on all three tested media. The conidial length and sporulation of Eml also differed significantly from all the other

isolates. The isolate Anm also showed differences in its growth rate when compared to the other isolates in two media (CDA and PDA). The conidial production and the conidial length were also proved to be significantly different for this isolate.

It is concluded that significant diferences exist among the isolates of *C. gloeosporioides* from banana. However, the differences do not occur in a regular pattern. The isolates, therefore, cannot be separated in to groups as reported for the *C. gloeosporioides* isolates from *Stylosanthus*.[3] The information obtained in this study should be useful in any future banana anthracnose management programmes.

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